Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

VOLUME XXXIII NUMBER 5

BOTANICAL GAZETTE

MAY, 1902

ON THE STARCH OF EVERGREEN LEAVES AND ITS RELATION TO PHOTOSYNTHESIS DURING THE WINTER.

KIICHI MIYAKÉ.

SACHS[1] suggested that in evergreen leaves the starch included in the chloroplasts might be stored there throughout the winter, but it seems that he did not make any observations of it. Mer,[2] by examining the contents of some evergreen leaves, found that the starch disappears at the end of October, to reappear again during the next March. Haberlandt[3] states that he found few starch granules in the palisade parenchyma of the leaves of *Taxus baccata* in winter.

Schulz[4] made a more extended study of the reserve materials of evergreen leaves in winter. He examined about a hundred species of plants, comprising both gymnosperms and angiosperms. All of the gymnosperms studied were found to be free from starch except *Gnetum Gnemon*, in which the green cells contained some starch-granules. Among the angiosperms all monocotyledons and some dicotyledons were free from starch, while the remaining dicotyledons contained more or less starch in the leaves. In many species, however, the starch was found only in the fibrovascular bundles and the surrounding tissues. It was only in *Viscum album* and *Euonymus japonicus* that the green cells of the leaves contained some starch. More recently Lidforss[5] in examining the leaves of several winter-green plants, comprising herbs, shrubs, and trees, came to the conclusion that all green cells of plants are entirely free from starch during the winter months.

---

[1] SACHS, J., Microchemische Untersuchungen. Flora **20**: 289. 1862. (p. 300.)

[2] MER, E., De la constitution et des fonctions des feuilles hivernales. Bull. Soc. Bot. France **23**: 231. 1876.

[3] HABERLANDT, G., Vergleichende Anatomie des assimilatorischen Gewebesystems der Pflanzen. Jahrb. Wiss. Bot. **13**: 74. 1882.

[4] SCHULZ, E., Ueber Reservestoffe in immergrünen Blättern. Flora **71**: 223. 1888.

It seems, therefore, according to the observations of the above investigators, that almost all of the evergreen plants of northern and middle Europe lose the starch from the green cells of the leaves during winter. To know whether a similar state of things holds true in Japan, and to throw some light on the relation of leaf-starch to photosynthesis, I undertook the present investigation at the suggestion of Professor M. Miyoshi, to whom my sincere thanks are due for kind suggestions and helpful criticisms. The work was carried on in the Botanical Laboratory of Tokyo Imperial University, from August, 1898, until June of the following year.

There are various methods of testing for starch, all using iodine as the principal reagent. After some trials I determined to use Meyer's iodine-chloral method.[6] The leaves to be examined were directly observed, or kept in alcohol (70 per cent.) until examined. When fresh leaves were used, several thin sections were made from each of them, and placed in strong alcohol until the decoloration was complete. They were then placed on the slide, a few drops of chloral-iodine and potassium-iodide solution[7] being added, and examined under the microscope. When alcoholic material was used, the sections were immediately placed on the slide for examination.

Among the climatic factors the temperature plays an important part in the process of photosynthesis, and it would not be superfluous, before entering upon the account of my researches, to make a short statement regarding the temperature of Tokyo and other parts of Japan during the winter.

---

[5] LIDFORSS, B., Zur Physiologie und Biologie der Wintergrünen Flora. Bot. Centralb. **68**: 33. 1896.

[6] MEYER, A., Das chlorophyllkorn. Leipzig, 1883.

[7] Prepared by mixing equal quantities of chloral-hydrate and water, to which a little iodine and potassium-iodide were added.

Tokyo is situated in about the middle portion of the main island of Japan, 35°59′ N., 139° E. In January, the coldest month of the year, the temperature at night often falls several degrees below zero centigrade, but rises sometimes to 10° C. during the day. The mean average temperature of the three winter months in Tokyo, taken from the observations of the last twenty years in the Central Meteorological Observatory, is as follows: December, 5°.1;[8] January, 2°.7; February 3°.5. The winter of 1898–99 was a little milder than usual, the record being as follows: December, 6°.4; January, 3°.2; February, 4°.2.

The following table shows the mean temperature of several stations from which the alcoholic specimens of evergreen leaves were sent to me for examination:

Station	Location	Long. and Lat.	Dec.	Jan.	Feb.
Kumamoto ..	Kiushu island, southern Japan	32° 48′ N. 126° E.	6°.2	4°.4	5°.6
Kyoto[9]	Middle Japan...........	35° 1′ N. 136° E.	4°.2	2°.3	2°.7
Ishinomaki[10]	Northeastern Japan.......	39° 16′ N. 139° E.	2°.7	−0°.8	0°.2
Sapporo.....	Hokkaido island, northern Japan..................	43° 3′ N. 138° 5′ E.	−3°.1	−6°.3	−5°.0

For comparison I give the mean temperature for January in several localities of Europe and America:

Berlin, − 0°.8[11]
Munich, − 3°.0
Hamburg, − 0°.4
Vienna, − 1°.7
Stockholm, − 3°.7
Kopenhagen, − 0°.4

London, 3°.5
Edinburgh, 3°.0
Dublin, 4°.7
Paris, 2°.0
Marseilles, 6°.4
Rome, 6°.7

New York, − 1°.0
Washington, 0°.2
Boston, − 3°.4
Chicago, − 5°.0
St. Louis, − 0°.5
Montreal, −8°.4

---

[8] Throughout the paper temperature is expressed in centigrade.

[9] No meteorological station was found at Nara, where the material was collected, so I give the record of Kyoto, about twenty miles from Nara, and probably with nearly the same temperature.

[10] For the same reason as above I give the record of Ishinomaki, about twenty-five miles from Sendai, where my material was collected.

[11] These figures were taken from J. Hann, Handbuch der Klimatologie. 1883.

STARCH CONTENTS OF EVERGREEN LEAVES DURING THE WINTER AND AT OTHER TIMES.

I first examined a number of evergreen plants, including angiosperms, gymnosperms, and pteridophytes, for the starch contents of the leaves in August. The results generally correspond to the investigation of Meyer.[12] Thus, generally speaking, monocotyledons have little or no starch in the leaves, while dicotyledons, gymnosperms, and pteridophytes are found to be fairly rich in starch.

Then, during several months, from August to the following spring, I examined about eighty evergreen plants for their leaf-starch, the material being usually taken twice or three times a month. The leaves for examination were taken from various parts of the plants, the healthy and full-grown ones being selected. In many cases they were taken from several individuals of the same species. To determine the starch contents four to eight cross-sections were made from a single leaf, and for such sections five to ten leaves were used. Of eighty species examined the following seventeen were found to lose the starch from the mesophyll during the coldest part of the winter:

Ilex rotunda.
Ilex latifolia.
Magnolia compressa.
Magnolia grandiflora.
Ternstroemia japonica.
Daphne odora.
Andromeda japonica.
Aucuba japonica.
Geranium acutarium.*
Asarum Blumei.*
Ephedra vulgaris.
Picea hondoensis.
Podocarpus Nageia.
Aspidium falcatum.*
Polypodium ensatum.*
Polypodium lineare.*
Gymnogramme japonica.*

* Herbaceous plants.

As it takes too much space to describe the results with all the species above mentioned, I shall give only those with *Ilex rotunda*, as a representative.

---

[12] MEYER, ARTH., Ueber die Assimilationsproducte der Laubblätter angiospermer Pflanzen. Bot. Zeit. **43**: 417. 1885.

ILEX ROTUNDA.

Date	Pal.[13]	Spon.	Stom.	M. t.[14]
1898, August 19	3-4[15]	3-4	II[16]	27.9
September 8	3-4	3-4	II	23.2
October 14	3-4	3	II	17.9
“ 20	3-4	3	II	13.0
November 7	3-4	3	II	10.9
“ 19	3	3	I	11.9
“ 24	2	2	I	7.0
December 2	2	2	I	9.7
“ 8	2	2	I	8.1
“ 23	1	1	O-I	−0.9
1899, January 9	1	1	O-I	3.5
“ 16	0-1	0-1	O-I	−1.2
“ 31	0	0	?	3.0
February 8	0-1	0-1	O-I	4.5
“ 27	2	2	I	8.5
March 6	3	3	I	8.9
“ 29	4	4	I	12.7
April 25	5	5	I	13.9

The following twenty-seven species were found to contain very little starch in the mesophyll during the coldest time of the year:

Citrus Aurantium Bergamia.
Ilex crenata.
Thea japonica.
Thea sinensis.
Thea Sasangua.
Eurya ochnacea.
Nandia domestica.
Berberis napalensis.
Myrica rubra.
Photinia glabra.
Rhaphiolepis japonica.
Laurus nobilis.

---

[13] The following abbreviations are used in the tables: *pal.*, palisade parenchyma; *stom.*, stomatic guard-cells; *spon.*, spongy parenchyma; *m. t.*, mean temperature. It was found that in many leaves there is some difference in the starch contents between palisade and spongy parenchyma, and hence I have denoted the starch contents of the two regions separately.

[14] The temperatures in this and other tables are taken from the observations made at the Central Meteorological Observatory of Tokyo, which is about one and a half miles from the Botanical Garden, where most of my material was collected. I found that there is very little difference in temperature between the two localities.

[15] In designating the relative quantity of starch in the mesophyll, the following numerical signs are used: *0*, no starch; *1*, very little starch; *2*, little starch; *3*, moderately rich in starch; *4*, rich in starch; *5*, very rich in starch.

[16] The starch in the guard-cells of stomata was examined at the same time, and the amount is shown in the following way: *O*, no starch; *I*, little starch; *II*, rich in starch; *III*, very rich in starch.

Osmanthus fragrans.
Trachelospermum jasminoides.
Hedera Helix colchica.
Dendropanax japonicum.
Quercus Vibrayeana.
Euonymus japonicus.
Thymus serpyllum vulgaris.
Rhododendron Metternichii.
Eleagnus pungens.
Eleagnus macrophylla.
Torreya nucifera.
Taxus cuspidata.
Cunninghamia sinensis.
Sciadopitys verticillata.
Tsuga diversifolia.

In the following table the results with *Thea japonica* are given

THEA JAPONICA.

Date	Pal.	Spon.	Stom.	M. t.
1898, August 19	3	3	1	27.9
September 8	3	3	1	23.2
“ 21	4	4	1	20.5
October 13	3–4	4	1	15.3
“ 21	2–3	3–4	1	17.3
November 7	2–3	3–4	1	10.9
“ 19	2	3	1	11.9
“ 24	1–2	3	1	7.0
December 8	2	2	1	8.0
“ 25	1	1	1	4.6
1899, January 9	1	1	?	3.5
“ 31	1	1	?	3.0
February 8	1	1–2	0	4.5
“ 27	2–3	3	0	8.5
March 13	2–3	4	?	4.1
“ 23	2–3	4	?	10.4
April 11	4–5	5	1	12.1
“ 23	4–5	5	1	15.3

The following twenty-one species were found to contain a little starch (2 in my scale) in the mesophyll during mid-winter:

Osmanthus aquifolium.
Actinodaphne lancifolium.
Gardenia florida.
Serissa foetida.
Rosa indica.
Pasania cuspidata.
Quercus glauca.
Illicium anisatum.
Fetsia japonica.
Skimmia japonica.
Ardisia japonica.
Pittosporum Tobira.
Ilex integra.
Saxifraga sarmentosa.*
Nasturtium officinale.*
Trachycarpus Fortunei.
Thuja occidentalis.
Chamaecyparis obtusa.
Abies firma.
Pinus densiflora.
Pinus Thunbergii.

* Herbaceous plants.

The results with *Osmanthus aquifolium* are as follows:

OSMANTHUS AQUIFOLIUM.

Date	Pal.	Spon.	Stom.	M. t.
1898, August 18	3	3-4	1	27.5
September 8	2-3	3-4	1	23.2
October 10	2	3	0-1	16.8
" 17	2	3	0-1	17.9
November 7	2	3-4	0-1	10.9
" 19	2	3-4	0-1	11.9
" 24	2	2-3	0	7.0
December 8	2	2-3	0	8.0
" 23	2	2-3	0	-0.9
1899, January 9	2	2	0	3.5
" 31	2	2	0	3.0
February 8	2	2	0	4.5
" 27	2	2-3	0	8.5
March 7	3	3-4	0	9.7
" 29	2-3	4	0	12.7
April 15	4	4-5	0	11.8

In the twenty following species, the mesophyll was found to be moderately rich in starch during the coldest period. They are the following:

Distylium racemosum.
Cinnamomum Camphora.
Viscum album.
Nerium odorum.
Daphniphyllum macropodum.
Eriobotrya japonica.
Juniperus chinensis procumbens.
Cryptomeria japonica.
Thuja orientalis.
Podocarpus macrophylla.
Cephalotaxus drupacea.
Thujopsis dolabrata.

The results with *Distylium racemosum* are as follows:

DISTYLIUM RACEMOSUM.

Date	Pal.	Spon.	Stom.	M. t.
1898, August 18	4	4	1	27·5
September 8	4	4	1	23·2
October 21	3-4	4	1	17·3
November 16	3	3	0-1	11·2
" 24	3	3	0-1	7·0
December 8	3	3	?	8·1
" 21	3	3	?	4·1
1899, January 9	3	2-3	?	3·5
" 31	3	2-3	?	3·0
February 8	3	2-3	?	4·5
" 27	3-4	2-3	?	8·5
March 7	4	3	0-1	9·7
" 29	4	4	1	12·7
April 23	5	5	1	15·3

The following table shows the starch contents of *Cinnamomum sericeum* and *Litsea japonica* in autumn, winter, and spring. It will be noticed how rich in starch these two plants are during the winter.

Name	Date	Pal.	Spon.
Cinnamomum sericeum	October 6	3–4	3–4
	January 31	4	4
	April 25	5	5
Litsea japonica	September 7	4–5	5
	January 18	5	5
	April 25	5	5

Thus among eighty species examined, the amount of starch in winter varies greatly in different species, the relation of the starch contents to the number of species being as follows:

Starch contents - - -	0	1	2	3	4	5
Number of species - - -	17	28	21	12	1	1

The majority of the evergreen plants were found to contain more or less starch in the mesophyll during the winter, though the amounts are generally much smaller than those at other times of the year. Only about one-fifth of the whole number of species examined became entirely free from starch, while some species were quite rich in starch during the coldest time of the year.

It can be seen, moreover, that the amount of starch during the winter does not depend much on the family to which the plant belongs. Taking members of Lauraceae, for example: *Laurus nobilis* has very little starch in the mesophyll during midwinter; *Actinodaphne lancifolia* has a little more, *Cinnamomum camphora* is moderately rich, *Cinnamomum sericeum* is richer, while *Litsea japonica* is very rich in starch. However, the mesophyll of all the ferns that I have examined[17] was entirely free from starch in midwinter, although the guard-cells always contain some starch.

Generally speaking, the amount of starch in the mesophyll begins to decrease in November and reaches its minimum during

---

[17] Besides the species of ferns already mentioned, I have examined *Aspidium varians*, *A. tripteron*, *A. lacerum*, *Pteris serrulata*, and *Lygodium japonicum*, in the coldest time of winter.

January and the beginning of February, increasing again from the end of February. As in the mesophyll, the amount of starch in the guard-cells becomes less or even entirely disappears in winter. Among the plants examined, less than half were found to have the stomata free from starch during midwinter. The increase or reappearance of stomatic starch was found to occur later than that in the mesophyll.

It should be noticed here that the starch contents of evergreen leaves are generally greater in April than in August or September. This fact was already noticed by Mer,[18] who remarked that some unknown internal causes may have an influence on the formation and accumulation of starch besides the known factors.

Lidforss (*l. c.*) noticed that the leaves are quite free from calcium oxalate crystals in winter, and he believes that this is favorable to the economy of plant life, since otherwise they would afford centers for ice-formation within the cells. Although not directly connected with my main object of study, I have examined carefully for the presence and amount of calcium oxalate crystals in the leaves of eighty evergreen plants at different times during the winter. I found the crystals in the majority of dicotyledonous leaves examined, and their amount did not decrease appreciably in winter.

Besides the plants already mentioned, I have examined some green herbaceous plants in the winter time. The results are as follows:

Name	Date	Pal.	Spon.	Stom.
Raphanus sativus	December 25	1	1	I
Raphanus sativus	January 18	1–2	1–2	I
Lamium album	February 12	0	0	I–II
Lamium amplexicaule	January 18	0	0	I
Senecio vulgaris	February 13	0	0	I
Campanula media	January 16	0	0	O
Stellaria media	January 18	1	1	?
Capsella Bursa-pastoris	January 18	2–3	2–3	I–II
Cynara Scolymus	January 18	0–1	0–1	I

---

[18] MER, E., Influence de quelques causes internes sur la présence de l'amidon dans les feuilles. Compt. Rend. **112**:248. 1891.

As is shown in the above table, some herbaceous plants contain no starch in the mesophyll in winter, while others retain some. In many of the herbaceous leaves a little starch was found in the guard-cells of stomata.

Some monocotyledonous plants which contain very little or no starch in the mesophyll, but much in the guard-cells during milder temperature, were also examined in winter, and more or less starch was always found in the stomata, as is shown in the following table:

Name	Date	Pal.	Spon.	Stom.
Iris japonica	December 5	0	0	II
Ophiopogon japonicus	January 7	0	0	I
Rhodea japonica	January 7	0	0	III
Aspidistra lurida	January 7	0–I	0–I	II–III

Thus we see that Lidforss's conclusion (*l. c.*) that the green cells of the plant, as well as the guard-cells, are entirely free from starch during winter months does not hold about Tokyo.

I have also examined many evergreen leaves collected in different parts of the country in winter and preserved in alcohol. The results are given below.

Evergreen leaves from Sapporo.

Through the kindness of Professor Miyabe of Sapporo Agricultural College, I have obtained alcoholic specimens of more than thirty species of evergreen leaves of that place, collected on January 22 and 25, and February 16. Among them the mesophyll of the following species was found to be free from starch:

Sciadopitys verticillata.
Pinus Thunbergii.
Pinus Strobus.
Pinus austriaca.
Pinus densiflora.
Picea excelsa.
Abies firma.
Abies sachalinensis.
Thuja orientalis.
Thujopsis dolabrata.
Podocarpus chinensis.
Taxus cuspidata.
Carex rhynchophylla.
Viscum album.
Ilex crenata.
Euonymus japonicus radicans.
Rhododendron brachycarpum.

The following contained little or very little starch in the mesophyll:

Cryptomeria japonica.
Pinus pentaphylla.
Pinus pumila.
Pinus ponderosa.
Picea ajanensis.
Juniperus chinensis.
Chamaecyparis obtusa.
Chamaecyparis obtusa breviramea.
Chamaecyparis obtusa pendula.
Chamaecyparis pisifera.
Chamaecyparis pisifera plumosa.
Ilex latifolia.

Hence we may say that in Sapporo the mesophyll of the majority of evergreen plants is almost free from starch during the coldest period of the winter. The guard-cells were also found to be almost without starch in the majority of the plants examined, about seven or eight species containing a little starch. Five species of dicotyledonous plants were found to contain the crystals of calcium oxalate in the leaves.

Evergreen leaves from Sendai.

The materials for study were sent to me through the kindness of Professor Yasuda of the Second Higher School of Sendai. They were collected on January 21 and February 13, 1899.

Of twenty-three species examined the following fourteen were found to contain no starch in the mesophyll:

Pinus densiflora.
Chamaecyparis pisifera filifera.
Sciadopitys verticillata.
Arundinaria Simoni.
Aucuba japonica.
Iris tectorum.
Rosa indica.
Nandia domestica.
Ilex crenata.
Andromeda japonica.
Euonymus japonicus.
Quercus glauca.
Photinia glabra.
Brassica campestris.

The following contained little or very little starch in the mesophyll:

Abies firma.
Cryptomeria japonica.
Thujopsis dolabrata.
Chamaecyparis obtusa.
Chamaecyparis obtusa breviramea.
Ilex latifolia.
Quercus Vibrayeana.
Thea japonica.
Thea Sasangua.

We may say that in Sendai also the mesophyll of the majority of evergreen plants is almost free from starch during midwinter. Guard-cells were free from starch in the majority of the examined plants, only five or six species containing a little starch. Calcium oxalate crystals were present, more or less, in almost all the dicotyledonous leaves examined.

Evergreen leaves from Nara.

The material was sent to me through the kindness of Mr. Aizama of the Nara High School, being collected in the cold days of January and February, 1899.

The results of the examination nearly correspond to those obtained from the plants of Tokyo. Among thirty-six species examined the following six contained no starch in the mesophyll:

Podocarpus chinensis.
Maesa Doraena.
Vicia Faba.
Eurya ochnacea.
Ternstroemia japonica.
Symplocos japonica.

Among the rest the following plants were found to contain little or very little starch in the mesophyll:

Podocarpus Nageia.
Pinus densiflora.
Pinus Thunbergii.
Nandia domestica.
Quercus glauca.
Citrus Aurantium Bergamia.
Rosa indica.
Photinia glabra.
Eriobotrya japonica.
Thea sinensis.
Thea Sasangua.
Thea japonica.
Eleagnus pungens.
Daphniphyllum macropodum.
Raphanus sativus.
Osmanthus aquifolium.
Ilex rotunda.
Ilex Oldhami.
Eurya japonica.
Illicium anisatum.
Ficus foveolata.
Raphanus sativus.
Osmanthus fragrans.

The following eight species were more or less rich in starch in the mesophyll:

Abies firma.
Cryptomeria japonica.
Nerium odorum.
Fetsia japonica.
Cinnamomum Loureirii.
Cinnamomum Camphora.
Cinnamomum pedunculatum.
Ardisia japonica.

About one-half of the plants contained some starch in the guard-cells, while calcium oxalate crystals were found in many leaves.

Evergreen leaves from Kumamoto.

The alcoholic specimens of evergreen leaves were sent to me through the kindness of Professor Aida, of the Fifth Higher School in Kumamoto. The leaves were collected at Kumamoto and its vicinity about February 20.

Of thirty-three species examined, starch was absent from the mesophyll of the following three :

Quercus glauca.
Quercus gilva.
Podocarpus macrophylla.

All the rest contained more or less starch in the mesophyll, and the following (more than one-third) were found to be rich or moderately rich in starch.

Citrus Aurantium Bergamia.
Cinnamomum Camphora.
Cinnamomum pedunculatum.
Daphniphyllum macropodum.
Gardenia florida.
Eriobotrya japonica.
Cryptomeria japonica.
Podocarpus chinensis.
Chamaecyparis obtusa.
Abies firma.
Pinus Thunbergii.

The remaining eighteen contained little or very little starch in the mesophyll, and are as follows :

Ilex latifolia.
Ilex integra.
Ilex crenata.
Ilex Oldhami.
Citrus Bigaradia.
Thea sinensis.
Thea japonica.
Euonymus japonicus.
Quercus sp.
Rosa indica.
Ligustrum japonicum.
Raphanus sativus.
Osmanthus fragrans.
Symplocos japonica.
Pasania cuspidata.
Thujopsis dolabrata.
Pinus parviflora.
Juniperus rigida.

Crystals of calcium oxalate were found in many leaves. It must be noticed that these results do not represent exactly that of the coldest time of the year in Kumamoto, as all the materials examined were collected in the latter part of February.

FORMATION AND TRANSLOCATION OF STARCH IN WINTER.

As stated before, many of the evergreen leaves in Tokyo contain more or less starch in winter. A series of experiments was performed to decide whether this starch is the product of photosynthesis going on in winter, or that which was formed earlier and kept there in the mesophyll without translocation. The experiments were mostly made with pot-plants, plants growing in the ground out of doors sometimes being used. The following plants were used in the experiments:

Thea japonica.
Fetsia japonica.
Rosa indica.
Andromeda japonica.
Cinnamomum Camphora.
Quercus Vibrayeana.
Eriobotrya japonica.
Hedera Helix colchica.
Ilex crenata.
Osmanthus fragrans.
Pinus densiflora.
Pinus Thunbergii.
Abies firma.
Podocarpus macrophylla.
Chamaecyparis obtusa breviramea.
Cryptomeria japonica.

The experiments were conducted between January 8 and the end of February. First of all, plants which contain more or less starch in the leaves were either put in the dark room [19] or covered with a black cylinder out of doors. After two or three weeks the leaves of most of the darkened plants were found entirely free from starch.[20] Then they were exposed to light. In most cases a small quantity of starch was formed after five to eight hours of exposure; in some cases it took ten to fifteen hours or more. The results of a few experiments are given below:

Thea japonica.

Two pot-plants were used, each about a meter high. On January 23, 1899, several leaves from each plant were examined for the starch contents and treated as below:

---

[19] During January and February, the temperature of the dark room was found to vary between 1°.0 and 7°.0 C.

[20] In some it took only ten days for the leaves to become free from starch, while in *Cryptomeria japonica* it took more than a month, probably due to the rich starch contents.

	Starch contents[21]			Treatment
A.	1-2	2-3	?	Put into the dark room.
B.	1-2	2-3	?	Remained out of doors as a check.[22]

At 9 A. M., February 10 (m. t. 3°.1, max. 8°.6, min. 2°.6) the leaves of both plants were examined, and it was found that the darkened plant had lost all of its starch in the leaves, while the starch of the check plant remained constant.

Then *A* was exposed to light out of doors. At 5 P. M. of the same day the leaves were again examined and the starch contents were found to be 1–2.

Fetsia japonica.

Two pot-plants, each about two-thirds of a meter high, were used. On January 23 the leaves of both were examined and treated as follows:

	Starch contents			Treatment
A.	3	3	I–II	Put into the dark room.
B.	3	3	I–II	Exposed to light out of doors as a check.

On February 17 the leaves of *A* were found to be free from starch, and the starch contents of *B* had remained constant. At 9 A. M. February 20 (m. t. 3°.4, max. 9°.7, min. −0°.8) the darkened plant was put into the light out of doors. At 5 P. M. of the same day the starch contents of *A* were 2, 2, I–II; and at 5 P. M. February 22 it became 3, 3, I–II.

Cinnamomum Camphora.

A small pot-plant about two-thirds of a meter high was examined for the leaf-starch at 2 P. M. February 9, and at once put into the dark room. The starch contents then were 2–3, 2–3. At 9 A. M. February 20, it was found that all the starch had disappeared from the mesophyll. The plant was exposed to light out of doors at noon of the same day, and at 5 P. M. of the same day the starch was found to be 1–2, 1–2, having been formed within five hours.

---

[21] The starch contents of palisade and spongy parenchyma, and of the guard-cells are denoted in three separate columns. It is moreover to be noticed that in *Thea japonica* the starch contents of small pot-plants are generally richer than those of the larger tree growing out of doors.

[22] In all experiments, the pots were buried in the ground to prevent the freezing of the earth.

Pinus Thunbergii.

Two pot-plants were used, each about half a meter high. On February 1 starch-contents of the leaves from both plants were examined and treated as below:

	Starch-content		Treatment
A.	3	?	Covered with a black cylinder.
B.	3	?	Check plant not covered.

The two put out of doors side by side.

On February 20 the starch had disappeared from *A*, and that of *B* had remained constant. At noon of the same day *A* was exposed to light, and at 5 P. M. the starch-contents were found to be 1–2, having been formed within five hours.

Abies firma.

Two pot-plants, each about a meter high, were examined for the leaf starch on January 23 and treated as follows:

	Starch-content			Treatment
A.	2–3	2–3	I	Put into the dark room.
B.	2–3	2–3	I	Remained out of doors as a check.

At 9 A. M., February 10, the mesophyll of *A* was found to be entirely free from starch. After exposure to the light out of doors, at 5 P. M. of the same day the starch was 1–2, 1–2, I; and at 5 P. M., February 11, the starch had increased to 3, 3, I.

In addition to these plants, several leaves of the following trees, which contain some starch in the mesophyll, were covered with tinfoil in the beginning of February: *Thea japonica*, *Quercus Vibrayeana*, *Sciadopitys verticillata*, *Osmanthus fragrans*, and *Ilex integra*. After two or three weeks the leaves were found to be entirely free from starch.

These experiments suffice to prove that many of the evergreen plants in Tokyo can form starch in the leaves by photosynthesis, and translocate it to other parts of the plant-body during the winter, though these processes may of course be much more feeble than at other times of the year. Hence the starch found in many evergreen leaves in winter may be considered as that which is formed during that season by photosynthesis.

BEHAVIOR OF STOMATA IN WINTER.

The continual exchange of gases between the intercellular spaces of the leaf and the surrounding atmosphere is necessary for photosynthesis, and this interchange is mainly through the stomata. Therefore, if the stomata be tightly closed, the photosynthetic process may practically be stopped in spite of favorable temperature and illumination.[23] Hence it would not be superfluous to note, the opening and closing of the stomata of evergreen leaves in winter, as observed in Tokyo.

Stahl[23] showed, in accordance with Schwendener[24] and Leitgeb,[25] that the stomata of evergreen leaves are closed during the winter, and also that the closed stomata of Taxus and Mahonia open after being kept for a week in a warm room, while those of Buxus and Hedera open after ten days. A similar fact was observed by Lidforss (*l. c.*) in the case of Saxifraga, Iris, and Lilium.

Darwin[26] verified the closure of the stomata of evergreen leaves in winter with his newly devised horn hygroscope. But he mentions some exceptional cases where they were found clearly open on January 20 (temperature 12°.5) in the warm winter of 1897–8, in *Prunus Laurocerasus*, *P. Lusitanica*, *Hedera Helix*, *Capsella Bursa-pastoris*, and *Cheiranthus Cheiri;* while *Buxus sempervirens* and *Ilex Aquifolium* had closed stomata.

For testing the opening of stomata I have adopted Stahl's cobalt test. The leaves, after removal, were brought immediately into the laboratory, the temperature of which varied from 15° to 17°, and covered with dried light-blue cobalt paper,[27] protected on both sides from the moisture of the air by two glass plates.

---

[23] STAHL, E., Einige Versuche ueber Transpiration und Assimilation. Bot. Zeit. **52**: 117. 1894.

[24] SCHWENDENER, S., Ueber Bau und Mechanik der Spaltöffnungen Monatsb. Akad. Wiss. Berlin, 833. 1881.

[25] LEITGEB, H., Beiträge zur Physiologie der Spaltöffnungsapparate. Mittheil. Bot. Inst. Graz **1**: 1886.

[26] DARWIN, F., Observations on stomata. Phil. Trans. Roy. Soc. London Bot. **190**: 531. 1898.

[27] Prepared by soaking the filter paper in 2–4 per cent. cobalt chloride solution.

At 1 P. M., January 24, 1899 (half cloudy, temperature outside 7°), four plants were examined. The results are as follows:

Name	Observed facts	Stomata
Ternstroemia japonica.......	Reddening of cobalt paper took place within a few minutes on the under side of the leaf, while it remained blue on the upper for a long time.	Distinctly open.
Thea japonica.....	Very faint reddening seemed to occur after some time, but it is doubtful.	?
Fetsia japonica......	Same as preceding.	?
Sciadopitys verticillata.....	No reddening occurred on either side.	Closed.

At 2–2:30 P. M. January 31 (clear, temperature 6°), the following plants were examined:

Name	Observed facts	Stomata
Raphiolepis japonica.	Young leaf — cobalt paper quite red after two minutes. Old leaf — no reddening after thirty minutes.	Distinctly open. Closed.
Ternstroemia japonica	Quite red after three minutes.	Distinctly open.
Osmanthus fragrans..	Reddening clearly seen after six minutes.	Open.
Sciadopitys verticillata	No reddening for a long time.	Closed.
Pinus densiflora......	Reddening after four minutes.	Open.
Quercus glauca......	Reddening clearly seen after four minutes.	Open.
Quercus Vibrayeana..	Reddening clearly seen after five minutes.	Open.
Quercus phillyraeoides	Reddening after five minutes.	Open.
Pasania glabra.......	Reddening after five minutes.	Open.

At 2 P. M. February 1 (rather clear, temperature 7°.5), the above four species of Fagaceae were examined again, and it was found that all of them had the stomata open as before.

The next day at 3 P. M. (temperature 8°), the following observations were made:

Name	Observed facts	Stomata
Thea japonica, *a*	Faint reddening after some time, but rather doubtful.	?
Thea japonica, *b*	Same as above.	?
Thea japonica, *c*	Reddening clearly observed within ten minutes.	Open.
Juniperus chinensis procumbens	Reddening after a few minutes.	Open.

It is to be noticed that in *Thea japonica* the leaves from the first two plants showed no distinct opening of stomata, while those from the third plant had open stomata. It seems, therefore, that there are some variations in the behavior of stomata of the same species, probably due more or less to the location of the plants.

It is evident that many of the evergreen leaves examined have stomata more or less open in day time during the winter.

SUMMARY.

1. The starch contents of evergreen leaves differ much at a given time according to the different species. Monocotyledons generally contain less starch than dicotyledons, gymnosperms, and pteridophytes, or even none in some species.

2. The starch in evergreen leaves, generally speaking, begins to decrease in November, reaching its minimum during January and the beginning of February, and increases again from the end of February.

3. Many evergreen leaves in Tokyo and other parts of middle and southern Japan contain more or less starch in the mesophyll, while it is entirely absent in some species in the coldest time of winter.

4. The starch found in evergreen leaves in winter is generally very little compared with that observed at other times of the year, but in a few species the starch is quite abundant.

5. This starch is formed by photosynthesis in winter, though feebly, and its translocation occurs in the same season.

6. The starch in the guard-cells becomes less or even entirely disappears in many species, while a few species contain a moderate amount throughout the winter.

7. The majority of evergreen leaves in the northern part of Japan nearly lose the starch from the mesophyll and guard-cells in winter, while a little starch is still found in some species.

8. The starch content of evergreen leaves is generally more abundant in spring than in late summer or early autumn.

9. The opening of the stomata in winter was observed in some evergreen leaves in Tokyo.

10. The entire absence of calcium oxalate crystals from the leaves in winter, as stated by Lidforss, could not be verified in the present investigation.[28]

CORNELL UNIVERSITY,
Ithaca, N. Y.

---

[28] A preliminary note, bearing the same title as this paper, was published in Bot. Mag. Tokyo **14**:44. 1900.